

EFFECTS OF ELEVATED PLATFORMS AND PERCHES ENRICHMENT ON BEHAVIOUR, OXIDATIVE STRESS, AND FEAR RESPONSES IN TURKEY POULTS

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ABSTRACT

A variety of stressors threaten commercial turkeys, resulting in physiological and behavioural alterations that negatively affect both welfare and productivity. Environmental enrichment (EE) is a potential strategy adopted to improve birds' life quality and enhance normal behaviour expression. Therefore, the current study aimed to evaluate the use of different EE tools for turkey poults, ensuring that these enrichments didn't cause stress and determining their efficacy in reducing the effects of potential environmental stressors and improving the birds' welfare. Randomly selected unsexed newly hatched one-day-old turkey poults were allocated into three floor pens with straw bedding for 7 weeks and classified into three treatment groups: Gp1 (C), control group, Gp2 (P), perches & hanging colored balls enrichment and Gp3 (TT), Elevated platform enrichment "turkey tree". Results revealed that implementation of EE increased walking and running frequencies in P and TT groups, respectively, and increased flying and wing flapping behaviour in both enriched groups, compared to the control. There were no statistically significant differences in malondialdehyde (MDA) levels, glucose levels, absolute or relative asymmetry, or mortality percentage, but enriched birds showed increased glutathione (GSH) levels. Fearfulness decreased, as indicated by shorter tonic immobility duration in the tonic immobility (TI) test, increased ambulation numbers, and reduced latency to ambulate, particularly in the TT group during the open-field test (OFT). This study demonstrated that providing physical EE objects can significantly benefit turkey poults' welfare by lowering fearfulness and increasing the expression of natural behaviours, supporting the importance of EE availability in early life.

Key words: Turkey, Enrichment, Stress, Fearfulness, Behaviour.

INTRODUCTION

The poultry industry has been one of the fastest-growing and most adaptable livestock sectors, playing a vital role in meeting the increasing demand for meat

and eggs and contributing significantly to national income (Rashad & Soliman, 2023). Turkeys represent an important component of this industry, playing a key role in meat and egg production. Recently, commercial turkey production has significantly increased (Erasmus, 2018) as a part of efforts to enhance the poultry industry and meet the growing demand for animal protein driven by the rising human population (Tona, 2018).

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A Wide range of stressors threaten commercial turkeys, including social stress, frustration due to increased bird densities (Lindenwald *et al.*, 2021), catching, transportation (Erasmus, 2018), unchanging environment (Newberry, 1999), feed restriction (Janczak *et al.*, 2007), cold (Zhang *et al.*, 2011), heat (Tsiouris *et al.*, 2018) stresses and pollutants. These stressors can result in physiological and behavioural changes, deteriorated egg and meat quality, increased mortalities, tissue and intestinal damage, and greater animal susceptibility to infections (Akinyemi & Adewole, 2021). Birds raised in such challenging conditions are more likely to panic and exhibit fear responses (Altan *et al.*, 2013), which negatively impacts both their welfare and productivity. The bird's ability to cope with environmental stress is crucial for successful poultry production as it is fundamental to effective health and welfare strategies. Enhancing birds' health could be achieved by a house that invokes less stress (Lindenwald *et al.*, 2021), including optimized management, feeding and housing conditions and environmental enrichment implementation (Kjaer and Bessei, 2013).

Environmental enrichment strategies (EE), such as elevated platforms, perches, and visual or tactile stimuli (e.g., colored balls), have been widely implemented to enhance poultry welfare and productivity (Lindenwald *et al.*, 2021; Jacobs *et al.*, 2023). These strategies support the expression of natural behaviours, stimulate activity, musculoskeletal development (Vasdal *et al.*, 2019), reduce fearfulness (Jacobs *et al.*, 2023), and enhance birds' coping ability in commercial environments (Altan *et al.*, 2013). Among these behaviours, perching, that considered a fundamental behavioural need for turkeys and an important welfare indicator, serving as a means for rest, refuge, and social spacing (Riber *et al.*, 2018; Bessei *et al.*, 2022). However, in the absence of adequate environmental complexity, turkeys may

redirect pecking behaviours toward conspecifics, leading to feather pecking or cannibalism (Bessei *et al.*, 2022), resulting in harmful outcomes such as feather pecking, vent pecking, and even cannibalism, which have both welfare and economic implications (Alemu *et al.*, 2016). In addition to behavioural assessments, physiological stress biomarkers such as malondialdehyde (MDA), an indicator of oxidative stress, and glutathione (GSH), a key endogenous antioxidant, offer valuable insights into systemic stress and animal welfare (Puvadolpirod & Thaxton, 2000; Surai, 2002; Lin *et al.*, 2006). Incorporating these biochemical measures allows for a more comprehensive evaluation of welfare under different enrichment conditions.

This study focused on three EE tools; Elevated platforms or turkey tree "TT", perch's "P" and colored balls were selected based on previous findings supporting their positive impact on poultry welfare and feasibility for use in commercial turkey systems. Elevated structures like TT and P were shown to stimulate perching behaviour and improve musculoskeletal health (Bailie *et al.*, 2013; Norring *et al.*, 2019), whereas colored balls offer visual and tactile stimulation that encourages exploration and reduces aggression associated with frustration (Jones & Carmichael, 1998; Tahamtani *et al.*, 2016; 2022). While EE strategies have been widely investigated in chickens (Estevez, 2009; Bailie *et al.*, 2013), their application and effectiveness in turkeys, a species with distinct behavioural and physical needs that differ from chickens in perching preferences, body structure, and social behaviour and may influence their interaction with enrichment tools, remain largely understudied. Notably, elevated platforms have been shown to reduce fear responses in chickens (Jacobs *et al.*, 2023), yet little is known about their impact on turkeys.

We hypothesized that the provision of different EE items, including perches, elevated platforms, and colored balls, would encourage bird activity, thereby improving the leg health and welfare of turkeys. Accordingly, this study aimed to evaluate the effects of those EE items on the welfare and behaviour of turkey poults, with particular attention to locomotion and behavioural reactivity. Additionally, the study was designed to ensure that the enrichment items did not induce stress and to assess their effectiveness in alleviating environmental stress, as indicated by selected biochemical markers and fear-related behaviours.

MATERIALS AND METHODS

This study was carried out in the turkey project, Beni-Suef Governorate, on the newly hatched turkey poults in the farm to examine the impact of physical environmental enrichment on behaviour and some stress parameters of turkey poults.

1. Ethical approval and welfare compliance,

The experiment was approved by the Institutional Animal Care and Use Committee (ICAUC), Beni-Suef University, number (022-250). All corresponding ethical guidelines were strictly followed, including those related to fasting duration prior to sampling, bird handling during rearing, isolation prior to behavioural testing, and non-invasive restraint methods during testing. To minimize stress, birds were habituated to human presence during daily husbandry routines. Behavioural assessments were conducted by the same trained personnel in a quiet room between 09:00 and 13:00 hours to reduce environmental and circadian stressors.

2. Physical environmental enrichment tools

2.1. Perches were designed according to González-Zapata *et al.* (2022). They

were gradually raised to allow birds to pass underneath and measured 5.5 cm (width) $\times$ 8 cm (height). To avoid damage to the birds' feet, the upper edges of the perches were rounded.

2.2. Balls, colored balls were suspended from overhead wires, hanging from the pen ceiling at a height of 30 cm above the ground at the beginning of the experiment, then they were raised by 5 cm each week (Jones *et al.*, 2000). The balls were 10 cm in diameter, red, yellow, blue, green, purple and pink in color and made of plastic.

2.3. Elevated platform "turkey tree, TT", consisted of three platforms of wooden framework, providing shelter and sitting areas at three different levels, located at 0.1, 0.7 and 1.3 m above floor level for the first, second and third platforms respectively. The lowest one measured 0.6 m $\times$ 1.6 m (0.96 m²), the 2nd platform measured 0.6 m $\times$ 1m (0.6 m²) while the 3rd one measurements were 0.6 m $\times$ 0.4 m (0.24 m²) (Lindenwald *et al.*, 2021). Three TTs were allocated evenly in the floor pen.

3. Birds' accommodation and grouping

A total number of 450 newly hatched one-day-old turkey poults with an average weight of 65 g were used. The temperature was set manually using an electric heater at 33°C for the first week. Then, the temperature was reduced by 2–3 °C gradually each week until reaching 24–25 °C. To ensure consistency and accuracy, the heater was calibrated prior to the experiment using a certified digital thermometer. Mixed-sex poults were randomly distributed into three floor pens each measured 10.67 m² with straw bedding for 7 weeks where they were classified into three treatment groups as following,

- Gp1 (C), Control group "receive no treatment, n=150 birds".

- Gp2 (P) Physical environmental enrichment with perches & hanging colored balls, n=150 birds.
- Gp3 (TT), Physical environmental enrichment with Elevated platform "turkey tree", n=150 birds.

All birds were reared under the same environmental conditions; ventilation was maintained using windows and exhaust fans. Both natural and artificial illumination were used over a total of twenty-four hours. Daily recording of the temperature and relative humidity levels (in the morning and evening) was done in each house using a digital thermo-hygrometer.

Water and feed are always available at any time and provided using manual plastic feeders and automatic drinkers. Bird feed was obtained from a feed factory affiliated with the Central Projects in the Governorate. The physical environmental enrichment tools were cleaned daily to remove fecal matter and reduce potential microbial load. All cleaning procedures were performed in accordance with standard hygiene protocols. Tool integrity was monitored throughout the study period to ensure safety and functionality.

4. Sampling

Based on a confidence level of 95% and a margin of error of 5%, the required representative sample size was calculated using a standard formula for finite populations (Krejcie & Morgan, 1970), resulting in a sample of 108 birds from a total population of 150 birds was considered in this study.

Since the only available data about the birds was their weight, stratified random sampling was applied using weight as the stratification criterion. The birds were grouped into three general weight categories: light, medium, and heavy. A final subsample of 30 birds was then randomly selected from the strata proportionally, ensuring balanced

representation across different weight groups (Cochran, 1977).

From this stratified sample of 30 birds, blood samples were collected from a randomly selected subsample of 10 individuals. This smaller subsample was considered sufficient for the biochemical analyses conducted, taking into account the exploratory nature of the study, as well as ethical and logistical constraints.

The ten randomly selected turkey poults from each group were fasted overnight, and then blood samples were taken from their wing veins. Weekly, samples were collected and divided into two clean and dry tubes. The first tube contained sodium fluoride, and the second one was empty. Samples were centrifuged for 10 minutes at 3000 r.p.m. in order to separate the plasma and serum. Plasma was used to determine the glucose content and serum samples were stored at -20°C for further biochemical analysis.

5. Measurements

5.1. Behaviour measurements

5.1.1. Behavioural observation

The behaviour of turkey poults was recorded twice a week throughout the experiment duration; birds were observed for 30 minutes. The Scan observation method was adopted at 60-second intervals according to Giersberg *et al.* (2020) and Ross *et al.* (2019). Kinesis and bird reactivity were estimated based on an ethogram described by Maria *et al.* (2004) and Adeleye *et al.* (2021) (Table 1).

5.1.1.1 Behavioural tests

Behavioural assessments were conducted during the 7th week of age. Both the tonic immobility (TI) test and open-field test (OFT) were performed between 09:00 and 13:00 hours to minimize the influence of circadian rhythms. The OFT arena was uniformly illuminated using standard white, fluorescent lighting (100 lux). Although no specific consensus exists regarding the

optimal lighting level for OFT in poultry, this level was chosen to provide adequate visibility while minimizing stress. This approach aligns with general

recommendations for maintaining consistent and non-aversive environmental conditions during behavioural testing (Forkman *et al.*, 2007; Jones, 1996)

Table 1: Ethogram of turkey poults.

Kinesis and locomotion	Walking	Walking forward, taking one or more steps.
	Running	Movement of the bird between two distant points at a higher speed than normal
	Flying	The flapping of the wings forces the bird to be lifted from the ground.
Reactivity	Wing Flapping	Extending both wings out from the body simultaneously and flapping of wings.
	Feather ruffling and body shaking	The neck is outstretched, feathers are snuffled and the whole body is shaken.
	Head shaking	Frequent shaking of the head

1.1.1.1. The tonic immobility (TI) test was conducted at the end of the experiment following the modified method of Noble *et al.* (1996). Ten randomly selected turkey poults were individually tested in a quiet, separate room isolated from environmental disturbances. Each bird was gently caught and placed on a flat stand in a lateral recumbent position. The assistant restrained the bird on its left side by placing the left hand over the right wing and gently grasping the legs with the right hand. After 15 seconds, the assistant gradually withdrew his hands. The bird was then observed from a distance of one meter, and the duration of tonic immobility (in seconds) was recorded.

The following parameters were assessed,

- One induction (OI%): the percentage of birds that entered tonic immobility on the first attempt.
- Vocalization (V%): the percentage of birds that were vocalized during the test.
- Defecation (D%): the percentage of birds that defecated during the test.
- Tonic immobility percent (TI%): the percentage of birds standing up voluntarily for the full 600 seconds without intervention.

- Tonic immobility duration (TID): duration of willingly standing up without being forced to do so.

Each behavioural response was quantified and expressed as a percentage of birds exhibiting the respective behaviour

1.1.1.2. Open-field test (OFT) was carried out based on the methods described by Erasmus and Swanson (2014) and Taskin *et al.* (2018). Ten randomly selected birds were tested individually in a neighbouring empty chamber adjacent to their home pen. The arena measured 1.5 m × 1.5 m with a concrete floor, and the lighting intensity was maintained at approximately 100 lux. A grid of 64 squares (each 0.04 m²) was marked on the arena floor with a permanent marker (Fig. 1). Each bird was placed gently in the center of the arena and observed for 10 minutes. The following behaviours were recorded: vocalization, ambulation, standing, sitting, defecation, and escape attempts. Additionally, latency to ambulate and latency to vocalize (both in seconds) were measured. For each behaviour, both the number of birds exhibiting the behaviour and the percentage of birds were documented (Table 2).

Table 2: An ethogram described birds' behaviour in the open-field arena.

Vocalization (V)	Production of sounds by birds
Ambulation (A)	Two or more treads in swift progression.
Standing	Standing on the floor of testing arena
Sitting (SI)	Sitting with breast and belly on the floor
Defecation (D)	Defecating of the animals during the test
Escape (E)	Endeavoring to leap out of the test stage



Fig. 1: Open-field arena for testing broiler turkey poults.

1.2. Developmental stability measurements,

After blood sampling, the measurements of morphological traits were taken, including right (R) and left (L) leg length (from the hock joint to the middle toe) and middle toe length (from the metatarsus to the nail). Both right and left values from one bird were taken during the same session. A digital calliper was used to record the length measurements in millimeters. The meaning of the right and left traits $[R+L]/2$ was used to calculate the trait size. The absolute differences between sides $[|R-L|]$ were used to define the fluctuating asymmetry of a trait. For all traits, relative fluctuating asymmetry was employed $[2|R-L|/(R+L)]$. The meaning of the relative asymmetries of the different traits has been referred to as combined relative asymmetry (Campo and Prieto, 2009).

1.3. Determination of biochemical parameters in turkeys' blood

1.3.1. Plasma glucose level was determined colourimetrically by the enzymatic reaction described by Young (2001).

1.3.2. Serum lipid peroxidation, a colourimetric estimation using the Albro *et al.* (1986) method of determining serum malondialdehyde (MDA) level, was adopted.

1.3.3. Reduced hepatic glutathione measurement (GSH), was carried out in accordance with Ellman (1959).

1.4. Bird's health monitoring and mortality recording:

Birds were monitored twice daily throughout the study period for general health, behavioural abnormalities, and mortality. Throughout the study, daily mortality was recorded, and overall mortality percentages were subsequently calculated. In the case of mortality, gross postmortem examinations were performed when feasible. No evidence of infectious disease outbreaks or clinical signs suggestive of systemic illness was detected. Most deaths occurred suddenly, without preceding symptoms, suggesting non-infectious causes such as accidental injury or failure to thrive. Therefore, all mortality data were retained in the final analysis, as no disease-related patterns unrelated to enrichment could be confirmed. Effect sizes for group comparisons were determined using Cohen's *h* (Cohen, 1988), and 95% confidence intervals were calculated using the appropriate formula as described by Bevans (2023) to ensure reliable interpretation of mortality outcomes.

2. Statistical analysis

The data were analyzed using SPSS version 22. Normality was assessed using the Shapiro–Wilk test, which is appropriate for small sample sizes ($n < 30$). For parameters with normally distributed data (Shapiro–

Wilk $P > 0.05$), one-way ANOVA was performed, followed by Tukey's post hoc test for multiple comparisons. Results were reported as mean $\pm$ standard deviation. For the vocalization latency and defecation number parameters, the Shapiro–Wilk test indicated a deviation from normality ($P < 0.05$), and therefore the Kruskal–Walli's test was used. These results were presented as medians with interquartile ranges (IQRs). Regarding mortality percentage, both statistical and practical significance were evaluated. Group comparisons were made using the Chi-square test. In addition to p-values, 95% confidence intervals (CIs) were calculated for each group in proportion to improve the precision of interval estimation. Furthermore, Cohen's h was computed to quantify effect size, with values of 0.2, 0.5, and 0.8 interpreted as small, medium, and large effects, respectively.

RESULTS

The TT group exhibited significantly increased walking frequency ($P < 0.05$) compared to the control (Fig. 2a). While the P group showed a higher running frequency compared to both the control ($P < 0.001$) and

TT ($P < 0.01$) groups. In addition, there was a significant increase in flying behaviour frequency in both (P), (TT) enriched groups ($P < 0.01$), compared to the control one.

Moving to the effect of EE on the turkey poult's reactivity, the results illustrated in Fig. (2b) proved that the frequency of wing flapping behaviour was significantly increased in enriched birds with perches and balls ($P < 0.001$) and TT ($P < 0.01$) as compared to control birds. Additionally, the P-enriched turkey poult's exhibited an increased ($P < 0.01$) wing flapping pattern than TT-enriched birds. On the other hand, results declared insignificant difference in feather ruffling & body shaking or head shaking behaviours between the three groups, despite the increase in the frequency of both behaviours in enriched groups ($P > 0.05$), particularly the P-enriched birds.

Regarding the effect of EE on some biochemical parameters in turkeys' blood results (Table 3), serum MDA and plasma glucose levels didn't significantly differ among the three groups, while serum levels of GSH revealed a notable decline in TT ($P < 0.01$) and P ($P < 0.001$) enriched groups, compared to the control one.

Fig. 2a: The effect of physical environmental enrichment on kinesis in turkey poult's

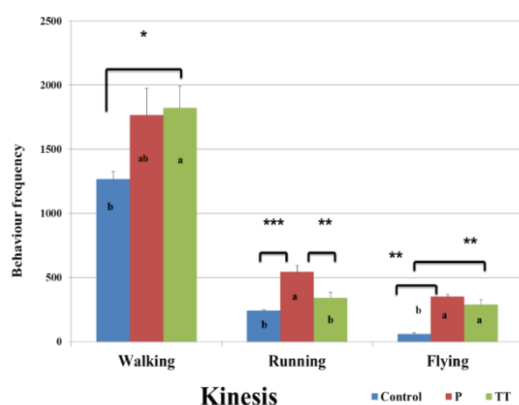


Fig. 2b: The effect of physical environmental enrichment on reactivity in turkey poult's

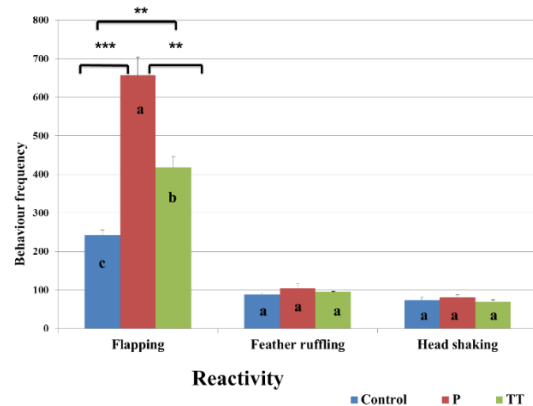


Fig. 2: The effect of physical environmental enrichment on behaviour of turkey poult's.

Results were expressed as means $\pm$ standard deviation

a,b,c litters indicate significance, the same litters indicate insignificance and * indicate significance values (*= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$)

P= Perches and balls.

TT= turkey tree.

Table 3: Effect of physical environmental enrichment on some biochemical parameters in the blood of turkey poult

Biochemical Parameters		MDA (mmol/l)	GSH (mmol/l)	Glucose (mg/dl)
Groups				
Control group.		0.13±0.00	0.32±0.00 ^a	329.48±5.81
Perches and balls (P)		0.14±0.01	0.23±0.01 ^b	326.95±7.26
Turkey tree (TT)		0.13±0.01	0.25±0.01 ^b	324.57±6.97
Enrichment tools to control	(P)	<i>P</i> =0.88	<i>P</i> <0.001	<i>P</i> =0.96
	(TT)	<i>P</i> =0.97	<i>P</i> <0.01	<i>P</i> =0.86
Between enrichment tools		<i>P</i> =0.76	<i>P</i> =0.22	<i>P</i> =0.96

Results were expressed as means ± standard deviation

Different superscript letters (a, b) within the same column indicate statistically significant differences between groups (*P* < 0.05).

TT= turkey tree, P= Perches and balls, NS= non-significant, MDA= malondialdehyde, GSH= reduced hepatic glutathione, mg/dl= milligram per deciliter, mmol/l =millimoles per liter.

Results of developmental stability were displayed in Table (4). They showed a statistically significant difference only in the size of the wing and leg traits. (*P*<0.01) of both P and TT enriched birds, where the insignificance in absolute and relative asymmetry ensures the stability in the

development of enriched birds and denies any adverse effect of physical environmental enrichment tools on the development of turkey poult during the growth period, but also positively affected the size of both wing and legs.

Table 4: Effect of physical environmental enrichment on developmental stability of turkey poult:

		Wing			Leg			Middle toe			Combined Abs. FA	Combined Rel. FA
		Trait Size	Abs. FA	Rel. FA	Trait Size	Abs. FA	Rel. FA	Trait Size	Abs. FA	Rel. FA		
Control		18.38 ± 1.18 ^b	0.35 ± 0.41	0.02 ± 0.02	6.81 ± 0.43 ^b	0.21 ± 0.23	0.03 ± 0.03	4.66 ± 0.22	0.32 ± 0.19	0.07 ± 0.04	1.76 ± 0.21	0.13 ± 0.07
P		20.05 ± 0.54 ^a	0.50 ± 0.47	0.02 ± 0.02	7.29 ± 0.17 ^a	0.22 ± 0.33	0.03 ± 0.03	4.92 ± 0.27	0.19 ± 0.16	0.04 ± 0.03	1.88 ± 0.20	0.08 ± 0.05
TT		20.05 ± 0.91 ^a	0.50 ± 0.41	0.03 ± 0.02	7.34 ± 0.34 ^a	0.29 ± 0.22	0.04 ± 0.05	4.64 ± 0.51	0.28 ± 0.20	0.06 ± 0.05	1.81 ± 0.23	0.12 ± 0.06
Enrichment tools to control	P	<i>P</i> <0.01	<i>P</i> =0.72	<i>P</i> =0.80	<i>P</i> <0.01	<i>P</i> =1.00	<i>P</i> =1.00	<i>P</i> =0.29	<i>P</i> =0.29	<i>P</i> =0.25	<i>P</i> =0.46	<i>P</i> =0.29
	TT	<i>P</i> <0.01	<i>P</i> =0.72	<i>P</i> =0.77	<i>P</i> <0.01	<i>P</i> =0.78	<i>P</i> =0.84	<i>P</i> =0.99	<i>P</i> =0.88	<i>P</i> =0.93	<i>P</i> =0.87	<i>P</i> =0.92
Between enrichment tools		<i>P</i> =1.00	<i>P</i> =1.00	<i>P</i> =1.00	<i>P</i> =0.95	<i>P</i> =0.83	<i>P</i> =0.84	<i>P</i> =0.22	<i>P</i> =0.53	<i>P</i> =0.40	<i>P</i> =0.76	<i>P</i> =0.46

Results were expressed as means ± standard deviation

Different superscript letters (a, b) within the same column indicate statistically significant differences between groups (*P*<0.05).

TT= turkey tree, P= Perches and balls, NS= non-significant, Abs.FA= absolute fluctuating asymmetry, Rel. FA= relative fluctuating asymmetry.

Regarding the behaviour of turkey poult in of arena (results in Table 5) revealed that the vocalization percentage and number didn't significantly change between the enriched and control groups. Nevertheless, the vocalization latency was increased ($P<0.05$) in TT and P ($P>0.05$) enriched birds as compared to control, while there was a significant increase ($P<0.05$) in vocalization latency in the TT- than the P-enriched groups.

Concerning the ambulation, its percentage didn't significantly differ among the three groups, but the ambulation number was considerably higher in TT-enriched birds, compared to the control ($P<0.001$) and P-

enriched ($P<0.01$) birds. Additionally, the TT-enriched groups exhibited a lower latency to ambulate in seconds when compared to the P-enriched and control groups ($P<0.001$). However, P-enriched birds had a longer latency period to ambulate "in seconds" than TT-enriched groups and control groups ($P<0.001$).

With regard to standing behaviour, neither its percentage nor number was affected by enrichment. Similarly, the number of birds sitting wasn't affected by enrichment, but the sitting percentage was significantly decreased in birds with TT enrichment than in those enriched with perches and control birds ($P<0.001$).

Table 5: The effect of physical environmental enrichment tools on turkey poult's behaviour in open-field arena

OFT Scoring Groups	Vocalization			Ambulation			Standing		Sitting		Defecation		Escaping		Square No.
	%	No.	Late ncy (S)	%	No.	Late ncy (S)	%	No.	%	No.	%	No.	%	No.	
Control	100	145.50 ± 32.40	3.50 ^b (2.25-4)	100	28.33 ^b ± 7.09	73.75 ^b ± 16.24	100	9.50 ± 2.22	16.67	0.17 ± 0.17	100	2.00 (2.00-2.75)	50 ^b	1.25 ± 1.25	22.75 ^b ± 4.79
Perches and balls (P)	100	167.00 ± 28.21	4.60 ^b (3.25-4.75)	100	29.50 ^b ± 6.65	159.0 ^a ± 23.47	100	8.60 ± 2.87	20	0.20 ± 0.20	100	3.00 ± (2.00-4.00)	60 ^b	1.75 ± 1.50	47.50 ^a ± 19.16
TT	100	137.00 ± 9.83	7.00 ^a (5-9)	100	84.25 ^a ± 9.46	3.50 ^c ± 1.29	100	12.71 ± 2.29	0	0.00 ± 0.00	85.71	2.00 ± (2.00-2.75)	85 ^a	2.25 ± 0.96	50.25 ^a ± 15.46
Enrichment tools to control	NA	$P=0.49$	$P=0.28$	NA	$P=0.70$	$P<0.001$	NA	$P=0.99$	$P=0.72$	$P=0.99$	NA	$P=0.32$	$P=0.20$	$P=0.84$	$P<0.05$
TT	NA	$P=0.89$	$P<0.05$	NA	$P<0.01$	$P<0.001$	NA	$P=1.00$	$P<0.001$	$P=0.64$	$P<0.001$	$P=1.00$	$P<0.05$	$P=0.52$	$P<0.05$
Between enrichment tools	NA	$P=0.27$	$P<0.05$	NA	$P<0.001$	$P<0.001$	NA	$P=0.99$	$P<0.001$	$P=0.57$	$P<0.001$	$P=0.32$	$P<0.01$	$P=0.84$	$P=0.96$

All results are expressed as means ± standard deviation, except for vocalization latency and defecation number, expressed as median and interquartile ranges.

Different superscript letters (a, b) within the same column indicate statistically significant differences between groups ($P < 0.05$).

TT= turkey tree, P= Perches and balls, %= percentage, S= seconds, NA= not applicable "Statistical analysis not applicable due to complete uniformity (100%) in observed behaviour".

Shifting to the defection behaviour, it was found that the percentage of birds defecating in the (TT) enriched group was significantly decreased, compared to the control and (P) enriched groups ($P<0.001$), despite the insignificance between the control and (P) enriched groups.

It appeared that the escape percentage was significantly increased in the TT-enriched group as compared to the control ($P<0.01$) and the P-enriched groups ($P<0.05$). Conversely, the escape attempts didn't significantly differ among the three groups, although it was the highest at the TT group ($P>0.05$).

Finally, the number of squares which poults were passed in the test arena was significantly increased in the (TT) group the (P) group ($P<0.05$) than in the control group, without any significant difference between the two enrichment groups.

The obtained result in Table (6), which pertained to the tonic immobility test revealed that the percent (%) of birds did one induction (OI) after the beginning of the test was

significantly decreased ($P<0.001$) in in TT enriched group, but was significantly increased ($P<0.05$) in P enriched group when compared to control one. Correspondingly, there was a significant increase ($P<0.001$) in OI% in the (P) than the (TT) enriched group.

Furthermore, there was a significant increase in the percentage (%) of birds did vocalization ($P<0.001$) in two enriched groups than control. Similarly, a significant increase in V% of the perches enriched group than the turkey tree enriched one ($P<0.001$).

Shifting to the defection percentage (%) among birds, there was a significant increase ($P<0.05$) in the perches enriched group only than the control one. Among the two enrichment groups, the defection percentage was significantly decreased ($P>0.05$) in the TT group than the P group.

Concerning the tonic immobility percentage (TI %), it was significantly decreased in both enriched groups than the control one ($P<0.001$). Additionally, there was no significant difference in the TI% between the two enriched groups.

Table 6: The effect of physical environmental enrichment on turkey poults' behaviour during the tonic immobility test.

TI parameters		OI (%)	V (%)	D (%)	TI (%)	TID (s)
Treatments						
Control group.		77.78 ^b	30.56 ^c	11.11 ^b	77.78 ^a	543.67 ^a ± 46.48
Perches and balls (p)		88.89 ^a	83.33 ^a	22.22 ^a	8.33 ^b	145.67 ^b ± 35.27
Turkey tree (TT)		55.56 ^c	58.33 ^b	11.11 ^b	8.33 ^b	32.17 ^c ± 8.06
Enrichment tools to control	(p)	$P<0.05$	$P<0.001$	$P<0.05$	$P<0.001$	$P<0.001$
	(TT)	$P<0.001$	$P<0.001$	$P=1.00$	$P<0.001$	$P<0.001$
Between enrichment tools		$P<0.001$	$P<0.001$	$P<0.05$	$P=0.60$	$P<0.001$

Results were expressed as means ± standard deviation

Different superscript letters (a, b, c) within the same column indicate statistically significant differences between groups ($P < 0.05$).

TT= turkey tree, P= Perches and balls, %= percent, S= seconds, NS= non-significant.

Finally results in Table (6) showed that the (TID) in seconds was significantly decreased in both (P) ($P<0.01$) and (TT) ($P<0.05$) enriched groups compared to the control, where the lowest duration was recorded in the TT enriched group. In contrast no significant difference

between the two enrichment groups, they had no significant difference ($P>0.05$).

The mortality proportions in Groups C, P, and TT were 13.4% (95% CI: 11.66%–15.14%), 11.93% (95% CI: 10.29%–13.57%), and

10.07% (95% CI: 8.56%–11.58%), respectively (Fig. 3). While there was a gradual decline in proportions from the first to the third group, a chi-square test of independence revealed no statistically significant differences among the groups ($p = 0.79$). Moreover, the 95% confidence intervals showed partial overlap, further suggesting that the observed differences may not be statistically meaningful. To assess practical significance, effect sizes were calculated using Cohen's h . The results were as follows: $h = 0.044$ between Groups C and P, $h = 0.093$ between Groups C and TT, and $h = 0.05$ between Groups P and TT — all below the threshold for a small effect ($h < 0.2$). These findings indicate that the differences in mortality proportions, while numerically present, are neither statistically nor practically significant.

DISCUSSION

Successful poultry production with minimal environmental stressors is the main goal of poultry producers. Hence, combating environmental stressors has become a great issue. Implementation of environmental enrichment was one of the main strategies that was implemented to decrease stress (Kjaer and Bessei, 2013).

By observing the turkey poult's behaviour after using EE, results revealed that there was an increase in the recorded different locomotor activities expressed by turkey poult's housed in an enriched pen, where TT showed a higher walking frequency, while running activities, a form of locomotor play (Gabrielle *et al.*, 2022), were the highest in the P group. Moreover, flying behaviour frequency was increased in both enriched groups, compared to the control. These findings were more or less similar to previous studies by Vasdal *et al.* (2019); de Jong *et al.* (2021); Mocz *et al.* (2022) and Jacobs *et al.* (2023), who declared that environmental enrichment stimulates activity, locomotion and play behaviour of birds, but disagreed with Baxter *et al.* (2020), who mentioned that play behaviours were similar in commercial broiler houses with or without different numbers of suspended platforms in broilers.

Likewise, Lindenwald *et al.* (2021) announced that the running and flying activity of turkeys was overall comparable between the EE and the control group.

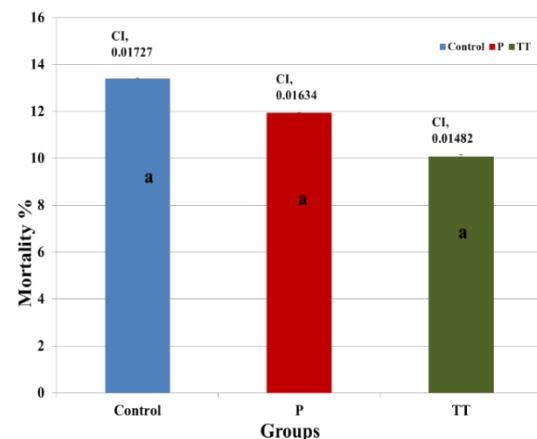


Fig. 3: The effect of physical environmental enrichment on the mortality percentage of turkey poult's. Results were expressed as proportion, Columns bearing the same letter are not significantly different ($p=0.79$). TT= turkey tree, P= Perches and balls, CI=confidence interval.

The significant increase in reactivity behaviour regarding wing flapping, which is also considered one of the locomotor play performed by birds, was in line with results obtained by Vasdal *et al.* (2019) and Elsayed *et al.* (2024).

The improvement in the behavioural activities of enriched turkey poult's may be attributed to the increased physical challenges accompanied with the presence of perches and an elevated platform (Norrington *et al.*, 2016). Platform enrichment provided birds with an elevated place where they can rest and perform the basic natural behaviour (Kaukonen *et al.*, 2017), increased the desire to move and the number of birds jumping, indicating a high motivation to use elevated structures (Elsayed *et al.*, 2024), which explained the more behaviour expression while using TT in the present study. Furthermore, the increased play behaviour regarding wing flapping and running indicated positive physical and affective states and lowered stress levels, as play typically occurs when stress is low (Burghardt, 2005). This was in accordance

with Anderson *et al.* (2021), who declared that environmental enrichment can potentially improve animals' cognitive development and elicit positive feelings.

Shifting to the effect of EE on some biochemical indicators of stress, there was insignificant change in MDA and glucose levels indicating that using of both perches and TT not only didn't pose stress on turkey poults but also, they contributed in lowering stress levels in agreement with Jiang *et al.* (2011), who mentioned a significant decline in breast muscle malondialdehyde in enriched broiler birds than in the control. El-Sabrou *et al.* (2024) announced that chicken productivity, welfare and health were maximized by implementing enrichment. So, EE is considered a crucial strategy for improving overall bird wellbeing and mitigating environmental stress.

The reduced GSH levels observed in the enriched groups could indicate increased oxidative stress, rather than a benefit from enrichment. However, the use of wooden physical enrichment tools in this study may have introduced confounding factors that contributed to GSH depletion. Specifically, such materials could increase the risk of microbial exposure (Moe *et al.*, 2010) and facilitate the accumulation of faecal matter on their surfaces (de Jong *et al.*, 2014), both of which may affect footpad condition, a variable that was not assessed in the present study and could have contributed to the lowered GSH levels. Prolonged exposure to dirty litter with high microbial load can lead to microbial decomposition and ammonia release, both of which are known to cause footpad dermatitis (FPD) and hypoxia. These conditions may result in systemic stress, even in birds with only mild lesions. Elevated ammonia and hypoxia have been shown to induce oxidative stress and disrupt various haematological parameters (Barus *et al.*, 2025). These considerations highlight the need for future microbial testing and more detailed investigations into the hygiene and microbial load associated with wooden EE structures.

Furthermore, while EE promoted increased locomotor activity, the associated biochemical findings point to a complex interaction between oxidative status and environmental variables, highlighting the need for comprehensive, multi-parameter studies in future research.

Physical asymmetry serves as an indicator for environmental stress throughout development (Dávila *et al.*, 2011 and Nelson *et al.*, 2020), as stress in poultry can cause asymmetry in all parts of the body (García, 2004). The insignificant change in the FA measures, except for the trait size of wing and leg, were in line with results obtained by Dávila *et al.* (2011), who found that the effect of physical enrichment on FA was not significant but disagreed with Nariç & Sabuncuoğlu (2022), who claimed that quails housed in conventional and enriched floor cages had noticeably reduced relative asymmetry values for wing length characteristics compared to those kept in traditional battery cages. Similarly, a study by Campo *et al.* (2008) found that the mean relative asymmetry of chickens grown in the alternative system was lower than that of hens raised in the conventional system. They also mentioned that environmental enrichment or alternative breeding systems reduced the deterioration of bilateral trait symmetry. The increased trait size of the wing and leg may be a reflection of musculoskeletal development due to increased bird activity as a result of enrichment (Vasdal *et al.* 2019) or due to improved performance.

Tonic immobility (TI) and open-field (OF) tests, validated tests for measuring fearfulness in poultry (Forkman *et al.*, 2007) as an indicator of stress (Ross *et al.*, 2020) and welfare (Papageorgiou *et al.*, 2023), highlighting how enrichment might mitigate negative emotional states (Anderson *et al.*, 2021). Results of this study denoted lowered fearfulness in the open-field test (OFT) for both enriched groups, particularly the TT enriched group, as evidenced by increased vocalization latency, decreased latency to ambulate and greater locomotor activity as indicated by a greater number of squares travelled. These findings contrast with

previous studies (Taylor *et al.*, 2022; Dumontier *et al.*, 2022), which reported no significant impact of EE on fear-related behaviours during the OFT. The combination of shorter ambulation latency and increased locomotion in the TT group suggests reduced fearfulness and enhanced exploratory confidence, as birds with lower emotional reactivity- i.e., those less prone to panic or stress when facing novel situations, typically showed increased exploratory behaviour in novel environments (Jones, 1996).

Interestingly, the increased escape attempts observed in the TT group during OF testing may not contradict these results but instead reflect enhanced exploratory tendencies and a stronger drive for social reinstatement drive rather than heightened fear. Enriched environments have been shown to promote physical activity, curiosity, and social interaction (Bizeray *et al.*, 2002; Ventura *et al.*, 2012; Zahoor *et al.*, 2022). Such behavioural variations may also stem from differences in the type of environmental enrichment used (e.g., platforms vs. perches) or discrepancies in testing protocols across studies.

Notably, Gallup and Suarez (1980) reported that more fearful birds were less likely to attempt social contact reinstatement, supporting the interpretation that the TT group's behaviour may indicate a motivated, socially confident profile rather than panic.

Moreover, the observed behavioural pattern can be interpreted within the framework of exploratory confidence versus social reinstatement motivation, which represents distinct behavioural systems. While lower fearfulness and increased locomotion indicate enhanced coping with novelty, escape attempts may represent an adaptive drive to rejoin conspecifics (Jones, 1996; Faure & Mills, 1993; Forkman *et al.*, 2007), indicating that the TT birds exhibited a proactive coping style rather than fear-induced behaviour.

Tonic immobility (TI) test results revealed longer TI duration, decreased TI% in both enriched groups, and decreased OI% in TT TT-enriched ones, indicating decreased

fearfulness. This was in agreement with Taylor *et al.* (2022), who mentioned that by implementation of EE using physical items could be a useful tool that can modify fear response in chickens. Furthermore, they reported that birds in more complex environments needed more attempts to induce TI, suggesting that providing physical EE items may decrease fearfulness after 21 days. In contrast, Bizeray *et al.* (2002), Dávila *et al.* (2011) and Taylor *et al.* (2022) mentioned that the TID, as an indicator of fear response, did not vary among birds given more complicated surroundings indicating that physical environmental enrichment alone may not be sufficient to reduce fear (Herrera-Alcaíno *et al.*, 2024).

The higher stimulation offered by an enriched environment that improved the animal's capacity to adjust to novelty (Jones 1982) may be the cause of the higher vocalization percentage in the TI test. In addition, EE lowered fear levels that caused an increase in the trials to reinstate social contact by birds. This possibly confirms the previous findings of Gallup and Suarez (1980), who emphasized that fear tends to inhibit calls by chickens.

The reduced fearfulness of enriched birds in TI and of tests could conclusively suggest that providing EE objects increased environmental complexity and solved welfare issues, such as reducing anxiety and fearfulness, (Mench 1998; Brantsæter *et al.*, 2017).

The disparity in behavioural response test results could be a consequence of different behavioural assessment timing, environmental habituation or shifts in social dynamics (Herrera-Alcaíno *et al.*, 2024). In addition, other factors may have influenced the birds' responses, like differences in handling, transporting and isolating situations of birds prior to testing and the social environment of testing, including individual or group testing as declared by Dumontier *et al.* (2022).

It was clear that the mortality rate wasn't significantly affected by EE despite the non-significant decrease in enriched groups.

Similarly, Mocz *et al.* (2022) and Busatta *et al.* (2024) mentioned that elevated platforms for broilers and hung colored plastic bottles as pecking objects for turkeys, respectively, have no significant effect on mortality. This disagreed with Balog *et al.* (1997), who mentioned that barrier EE decreased mortality in broiler birds.

Although EE yielded positive behavioural outcomes indicative of improved welfare, no significant reduction in mortality was observed. This may be due to the relatively short duration of the study (7 weeks) and the sample size, which may have limited the ability to detect statistically meaningful differences in survival. As such, the absence of a mortality effect should not be interpreted as a lack of welfare benefit, but rather as a limitation in the study of power and duration. Future research involving longer-term trials and larger sample sizes is necessary to better evaluate the potential impact of EE on survival outcomes.

LIMITATIONS

This study had several potential limitations. First, the experiment was conducted on a single farm due to the limited number of turkey farms in Beni-Suef Governorate, which may affect the generalizability of the findings. Second, the study followed an experimental design with a relatively short duration and a limited sample size, which may not fully capture long-term effects. Third, wooden EE tools were used primarily because of cost considerations and the assumption that regular cleaning would minimize microbial load. However, microbial factors were not measured directly, which limits our understanding of potential microbial influences. Further-more, the use of FPD scoring alone may provide insufficient evidence to conclusively explain the observed elevation in GSH levels associated with the use of wooden turkey tools (TT). These limitations highlight the need for future studies that evaluate the efficacy and microbial safety of wooden TT compared to metal ones and explore their effects across different bird ages throughout the rearing period.

CONCLUSION

Using EE, particularly elevated platforms during the early life of turkey poults, improved welfare in multiple aspects, including enhanced behaviour, reduced stress and fear responses, and lower mortality rates. Moreover, the reduction in stress biomarkers likely provides evidence that EE tools can be safely applied to turkey poults without causing additional stress. This study also suggests that a less complex environment may contribute to depression, frustration, stress, and reduced behavioural expression.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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آثار إثراء المنصات المرتفعة والمجاثم على السلوك، الإجهاد التأكسدي واستجابات الخوف في صغار الرومي

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يتعرض الرومي التجاري لمجموعة متنوعة من مسببات الإجهاد مما يؤدي إلى تغيرات فسيولوجية وسلوكية تؤثر سلباً على كل من الرفاهية والإنتاجية. يُعد الإثراء البيئي (EE) أحد الاستراتيجيات المتبعة لتحسين جودة حياة الطيور وتعزيز التعبير عن السلوك الطبيعي. لذا، تهدف الدراسة الحالية إلى استخدام أدوات الإثراء البيئي المختلفة لصغار الرومي لضمان عدم تسببها في الإجهاد وتحديد فعاليتها في تقليل تأثير مسببات الإجهاد المحتملة وتحسين رفاهية الطيور. تم توزيع صغار الديك الرومي حديثة الفقس وغير محددة الجنس والتي يبلغ عمرها يوماً واحداً بشكل عشوائي في ثلاثة عنابر أرضية مع فرشاة من القش لمدة ٧ أسابيع وتصنيفها إلى ثلاث مجموعات علاجية تتضمن مجموعة ١: الكونتروال "المجموعة الضابطة" C.gp. ومجموعة ٢: المجاثم والكرات الملونة المعلقة P. gp.)) "Perches and hung colored balls" gp. ومجموعة ٣: وإثراء المنصة المرتفعة "شجرة الديك الرومي (TT. gp. (elevated platform)). هذا وقد كشفت النتائج أن تطبيق الإثراء البيئي أدى إلى زيادة وتيرة المشي والجري في المجموعتين (P) و (TT) على التوالي، وأيضاً زاد من سلوك الطيران ورغبة الأجنحة في كلتا المجموعتين المعرضتين للإثراء البيئي مقارنةً بالمجموعة الضابطة. كما لم يكن هناك فرق إحصائي في مستويات كل من المالدنديالدهيد MDA، أو الجلوكوز، أو التماثل المطلق "FAA" أو النسبي "RFA" أو معدل الوفيات، ولكن كان هناك زيادة في مستويات الجلوتاثيون GSH في الطيور المعرضة للإثراء البيئي. كما انخفض مستوى الخوف، وقد تبين ذلك من خلال قصر مدة الجمود التوتري (TI) في اختبار الجمود التوتري TI، وزيادة عدد مرات المشي، وانخفاض فترة الكمون للمشي "latency to ambulate" وخاصةً في مجموعة TT وذلك في اختبار المجال المفتوح "OFT". أظهرت الدراسة الحالية أن توفير أدوات للإثراء البيئي المادية والذي يمكن أن يكون له فوائد كبيرة على صحة الرومي، بما في ذلك انخفاض الخوف وزيادة التعبير عن السلوك الطبيعي، مما يدعم أهمية توفير أدوات للإثراء البيئي في المراحل المبكرة من حياة الطائر. الكلمات المفتاحية: الرومي، الإثراء البيئي، الإجهاد، الخوف، السلوك.